VULNERABLE AND PROTECTED ENDEMIC SPECIES FROM THE PROTECTED AREAS OF BIHOR COUNTY. THEIR CONSERVATION THROUGH *IN VITRO* MULTIPLICATION

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Abstract:

In vitro culture of the rare and endangered species ensures the multiplication of these species and also the repopulation of the areas from where they come. Species that were the research object in this study and that were experimented come from the "Şes Mountain" protected area (SCI sit from Bihor) are: Dianthus spiculifolius (syn. serotinus) Schur. var. Transilvanicus (Fam.Caryophyllaceae) critically endangered specie (CR) and Drosera intermedia Hayne., (fam. Droseraceae) critically endangered specie (CR). The species have a scientific value and are geo elements with poor populations, rarities, and also of didactic and ornamental interest. The purpose of the experiment was the conservation and the protection of these three species through in vitro multiplication. The plant material used for the multiplication was formed of: juvenile flower bud (Drosera), meristematic tissue – apex (at Dinathus). Culture mediums were conceived in a balanced manner, with the mediums according to Murashige – Skoog, to which there were conceived three medium variants for each specie (Table 3) with hormones (an auxine and a cytokinin) in a concentration of 0,5 – 1,0 mg/l. There was followed the evolution of the explants after about 40-50 days of in vitro culture: aspects concerning the percentage of regeneration, multiplication, rooting and acclimatization, and also the number of the differentiated plantlets/explant.

For the in vitro multiplication of Dianthus specie, we recommend the MS medium simple or with a balanced hormonal addition (V_3), conditions which ensure the obtaining of some superior biological parameters (□90% regeneration; □50% acclimatization).

The Drosera bud has a favorable reaction to in vitro culture influenced by the presence of the cytokinins in the MS culture medium. On the witness sample MS (V_o) the regeneration is of 30%: on the variants with BA and Z 1 mg/l (V_1 and V_2) it is of 90 and 100%, and the average of the number of neoplantlets reaches even 28 – 38 plantlets/explant. We recommend for a successful multiplication of this species the presence of the cytokinins in the basal medium in a moderate dose of about 1 mg/l (especially Zeatin), on which there are forming plantlets completely organized and well rooted that acclimatize □ 45%. The research have proved that the spontaneous species rare and endangered lend to the in vitro multiplication with the purpose of their conservation and extending in the areas of origin, and also in the landscape architectural spaces, based on their natural and ornamental value.

Keywords: protected areas, conservation, biodiversity, critically endangered species (CR), vulnerable (VU), rare (R), *ex situ*, *in situ*, red list, red book of the plants: *Dianthus spiculifolius L., Schur. var. Transilvanicus; Drosera intermedia Hayne*, *in vitro* multiplication, *ex vitro* acclimatization, ecological reconstruction.

INTRODUCTION

The moderate action of the environmental factors over the plants makes so that they survive and mutual balance, but if a factor becomes predominant it can advantage some species or populations and can disadvantage others (Boşcaiu el all., 1994; Dihoru, and Negrean, 2009),

hence some species of plants becoming rare or sporadic this is why they present much more interest for the researchers even though they are not endemic. The number of rare species from Romania is large (due to the continuous area) some of them are located at the boundary of the area (Cristea, V., et all. 1996; Bleahu, 2004; Baciu et all., 2006). The phenomenon of the disappearance of the species has multiple causes and leads to the decreasing of the habitat, to the reduction of the natural reserves, to the degeneration of the environment and of some species, to the loss of deduction of the biological potential of the populations of plants, etc. (Flora RSR vol. XIII; Dihoru and Dihoru 1994). Hence the action of the conservation of the endangered species of plants is one of interest for specialized NGOs, and also for the conservatorist specialists from other fields, as it would be in our case through plant biotechnologies (Conferința ONU, 1992; Cachiță and Ardeleanu, 2009; Primack, 2001; Laslov, 2013). Through the Convention of Berne (1979) there were taken measures for the prevention of some dangers (of eco-filaxy) and also some concerning the development of the gene banks (IBPGR, 1986; Bavaru et all., 2007). According to IUCN, in 2006 the rhythm of the disappearance of the species of plants on the globe is of 100 up to 1000 times more intensified, man and his activity being the main cause of their extinction (according to IUCN one of 8 species of plants is threatened with extinction). It is estimated that in the last 50 years about 300.000 species have disappeared, and a percentage comprised between 20-40% from the global flora is ebbing away (Farusworth, 2008). At a European level, about 100 species from the threatened ones are included into *in situ* restoration programs, and about 35% from the taxons are under a program of minimal protection (De Langhe, 1984; Maunder, Higgens 1998). *In situ* conservation means monitoring – protection plans of the habitats where there the endangered species of plants can be found (Cristea, 2006; Domuța (editor) in: Breja et all., 2013). There are recovery plans even for one single endangered specie, and also *ex situ* conservation activity (Bajaj, 1986; Halmagyi and Butiuc-Keul, 2007; Blându and Holobiuc, 2008).

In the conservation of the species of plants the alarm signal and the information one over the sezological estate of the species belongs to the elaboration and the update of the "red lists" and of the "red Book" (Olteanu et all., 1994; Dihoru and Dihoru, 1994; Boșcaiu et all., 1994; Dihoru and Negrean, 2009), which encompass the endangered species from the entire country. The conservation of those species through unconventional methods: *in vitro* presents a great interest and have a future ahead (Engelman, 1997; Zăpârțan, 2001). The method was experimented at the horticultural species and also to the botanical elements with a scientific value from the spontaneous flora (Engelman, 1991; Fay, 1992: Zăpârțan

1995; Laslo et all., 2011a; 2011b). The advantages of this method are multiple, but we insist on the advantage that it concentrates on the fact that in the initiation of the culture it is necessary a single plant (Cachiță, 1987), a seed, a single explant (the peak of the sprout, the flower bud, a portion of the leaf and of the stem, etc), so these plants that are so few, will not be affected by their harvest from their place of origin (Zăpârțan, M, 1996, 2002).

The research concerning in vitro techniques at the rare endangered and endemic species from Romania, for their conservation were applied at a large number of species (Zăpârțan, 2001), some obtained results were presented at international symposiums (Zăpârțan, 1994, 1996). The implication of the plant biotechnologies in the multiplication of some species of plants that multiply with difficulty through the classical method, are much older and continued until nowadays (Cachiță and Ardelean, 2009; Laslo, 2013). The field extended encompassing *in vitro* photoautotroph cultures at vulnerable and endangered species (Cristea, Victoria, 2010) and even at some taxons of Dianthus, rare and endemic (Cristea, Victoria., et all., 2004; Zăpârțan, M., 1995), also threatened with extinction.

MATERIAL AND METHODS

In Bihor County there are over 64 natural reserves and monuments of nature established until the 1st of May 2007 on a surface of 30.867 hectares from which 30.545 hectares belong to the Apuseni Natural Park (Bavaru et all., 2007). These reservations and monuments of nature from Bihor County are of botanical, palaeontological, speological, zoological, geological and mixed nature. The present study had as a purpose *in vitro* conservation of the following species from the protected areas of Bihor County: *Dianthus spiculifolius (syn. serotinus) Schur. var. Transilvanicus (Fam. Caryophyllaceae) and Drosera intermedia Hayne., (fam. Droseraceae),* all of them coming from the Şes Mountain protected area, a site of community importance (SCI). Table 1 presents the three species researched *in vitro*, their popular denomination, their importance, location and sozological status. Some endangered species from the area of Bihor and from the surrounding areas were successfully conserved *in vitro*, hence we recall the rare species form Piatra Craiului Mountain, Gilău, etc. (Cristea, V, at. all., 2004; Blându, Holobiuc., 2007).

Table 1

Floristic species cultivated *in vitro* from the protected area of Şes Mountain (SCI sit) from Bihor County

Specie of floristry rarity	Popular name	Importance	Location	Sozological status
Dianthus spiculifolius (syn. serotinus) Schur. var. Transilvanicus (Fam.Caryophyllaceae)	Sweet William	*Scientific interest; Dacian – Pontic endemic, small area and poor populations. Ornamental plant.*	Şes Mountain (SCI sit):	CR = critically endangered
Drosera intermedia Hayne., (fam. Droseraceae)	Sundew	*Scientific interest; Dacian – Pontic endemic, small area and poor populations. Ornamental plant.*	Şes Mountain (SCI sit):	CR = critically endangered

The chronology, habitat, area, biology and taxonomy of the studied species. *Dianthus spiculifolius (syn. serotinus) Schur. var. Transilvanicus*, signaled in the flora of Romania in 1953 (Prodan 1953), now sporadic present over the Someş, Prut, Olt Defile (on sends and loess), and also in a few other points from Transylvania (Boşcaiu at all., 1884). A geo-element with a sozological status of critically endangered plant, important form a scientific point of view by the fact that it is Dacian – Pontic endemic with a restricted area and with very poor populations, protected where it is found in reservations (e.g. Râpa Roşie), and also in botanical gardens or as a germoplasma in the gene banks.

Drosera intermedia Hayne, insectivore specie from the *droseraceae* genre met only in Transylvania (Ţopa, 1955; Gişa., Gergely 1963; Web 1993). Drosera has a sozological status of critically endangered plant (CR), it is surely found in Gilău Mountains, Great Mountain, Şoimului Springs in the swamps (Ţopa, quoted by Dihoru and Negreanu 2009). Circumboreal element, European geo-element with a cenologic habitat in swamps of peat oligotrophic, glacial relict (Popa 1960), in many areas from Transylvania they have become rare after the arrangement of some pastures (Täuber, Weber, 1976), hence the area of the specie is restricted with a habitat specific to the South-East limit of the European area and in our country with populations concentrated in Gilău Mountains and in the surroundings, but disappeared from other areas. It presents a scientific interest as a biological rarity (insectivore specie). *In situ* it is conserved in Gilău Mountains, acclimatized and cultivated in the greenhouses of the botanical gardens from the country, and the germoplasma in the gene banks.

The *plant material* used for the *in vitro* culture of the species mentioned above was composed of young floral bud of about 2mm Ø (at Drosera), apical meristematic tissue or apex (at Dianthus). The types of explants used, basal mediums, the time of the year when the experiment was initiated and the duration of tissue incubation *in vitro* are presented in Table 2.

Table 2

Types of tissues and the culture mediums used in the *in vitro* multiplication of the studied species

The specie experimented *in vitro*	**Basal medium (MB)**	**Type of experimented tissue**	**The time of the year when the experiments were initiated**	**Duration of the experiment (days)**
Dianthus spiculifolius (syn. serotinus) Schur. var. transilvanicus	MS	Apex, apical meristem	March – April	30 – 40
Drosera intermedia Hayne.	MS	Young floral bud of about 2mmØ	August – September	55 – 65

. The plant material was harvested from the protected area with great care in order not to affect the species that are already in a small number, being present in a sporadic manner and in poor populations. The plant material with which was initiated the *in vitro* culture was harvested there where the species are in a larger quantity and only a single explant that was planted in the flowerpot in a cold greenhouse (considered mother plant donor of explants); then we could resort to the sampling of a single inflorescence or sprout (from the plant from that area) from which there was incised a young bud or apexes from the sprout, without affecting the plant, operation that must be done in the day of the experiment or the day before (for keeping the properties of the tissue). After the sterilization of the material, is sectioned and inoculated on the ***basal medium (MB)***:, the *apex* of *Dianthus* and the sectioned *bud of Drosera* was inoculated on the MS (Murashige – Skoog, 1962): for each specie conceiving a few medium formulas with hormonal addition and supplemented with other substances specified in Table 3.

Table 3

Medium formulas used for the in vitro multiplication of the species from the Şes Mountain area

SPECIE	**Var.**	**MB**	**AIB mg/l**	**BA mg/l**	**Z mg/l**	**Additional additives gr./l or mg/l**
Dianthus	V_0	MS	-	-	-	-
	V_1	MS	-	-	-	3g/l vegetal coal (CV)
	V_2	MS	0.5	1.0	-	825mg/l NH_4NO_3
	V_3	MS	0,5	-	1.0	
Drosera	V_0	MS	-	-	-	-
	V_1	MS	0.5	1.0	-	-
	V_2	MS	0.5	-	1.0	-
	V_3	MS	0.5	0.5	-	-

(**MS** = Muraschige-Skoog; **AIB** = indolyl butyric acid*;* ***BA*** = benzyl adenine; **Z** = Zeatin)

Incubation conditions of the in vitro cultures. After inoculation on the aseptic mediums the explants were kept in the conditions of the growth chamber, at a luminous intensity of 16 hours light from 24 hours, at a temperature of about 26^0C and a humidity of about 80%. The light and its intensity varies depending on the purpose and on the specie, being used a

diffuse Fluorescence light with an intensity of 2-10 klux, depending on the stage of development of the neoplantlets, then there was used a continuous light or a mixture Fluorescence light with red – violet, necessary to some types of inoculums in order to induce the organogenesis. Then there are situations when there it is applied a period of dark (4-6 days) during which within the bud there is triggered the floral induction; or locating for a few days (also 4-5 days) the callus in the dark for stimulating the hormonal activity within the tissue. There are species that need during the period of incubation another light, temperature and humidity regime, using in this case acclimatized closets capable of ensuring the desired conditions of temperature and photoperiod.

RESULTS AND DISCUSSION

After the inoculation of the explants on the mentioned aseptic mediums, the phials were kept in the conditions of the growth chamber recalled above, and after 40 respectively 50 days there were made measurements and observations concerning: the percentage of regeneration, of rooting, of multiplication and of acclimatization of the explant of Campanula and Drosera bud and of the apical tissue (apex) at Diantus, and also the average of the number of neoplantlets regenerated from each explant. Table 4 encompasses the values of the observed parameters (the average of the number of differentiated plantlets, and also the percentages of regeneration, multiplication and acclimatization).

Table 4

The values of the analyzed parameters at the three species cultivated *in vitro* (after 40-50 days)

No. crt.	The experimented specie/explant	Var.	% Regen.	No. pl./expl.	% Rooting	% Multip	% Acclim
1.	*Dianthus spiculifolius var. Transilvanicus* - apex	V_0	11	2	10	20	20
		V_1	54	16	20	38	25
		V_2	68	21	23	60	44
		V_3	**98**	**28**	**25**	**100**	**52**
2.	*Drosera intermedia Hayne.* - bud sectioned of about 0,2mm Ø	V_0	32	2	10	2	2
		V_1	90	28	30	80	32
		V_2	**100**	**38**	**30**	**100**	**42**
		V_3	70	20	29	60	30

The observations have proved that the *in vitro* regeneration process of the tissue detached from the species from Şes Mountain, follow the natural biological cycle of the specie and that during the time of the year when the plant has the highest capacity of *in vitro* regeneration it is influenced both by the climate conditions and by the time of the year when the experiment is developed and by the nature of the specie. The favorable time of the

classical multiplication of the species of plants (for the multiplication) is early spring for most perennial or annual species and late autumn (the end of November) for the ones that multiply through bulbs (Floriculture), for the *in vitro* culture it was proved that there are favorable the same periods of time (Zapârţan, 2001).

The presentation of the results of the experiment was done for each of the species from which then there were formulated conclusions and general recommendations.

I. The evolution of the explant from the apical tissue (apex) of *Dianthus spiculisolius var. Transilvanius* at the *in vitro* culture. Dianthus specie multiplied *in vitro* from apex regenerates in a percentage of 98% in the presence of Zeatin, multiplies 100% and is acclimatized to the *ex vitro* conditions in a good percentage of over 50% (Fig. 1). On all variants with phytohormones takes place a good regeneration and multiplication (determined by the dose and the nature of the phytohormone). The average of the number of plantlets differentiated form an explant is comprised between 16-28 plantlets/apex (Fig. 2), and at this specie, the evolution of this parameter depends on the presence or on the absence of the auxins and cytokinins (on the used formula of medium).

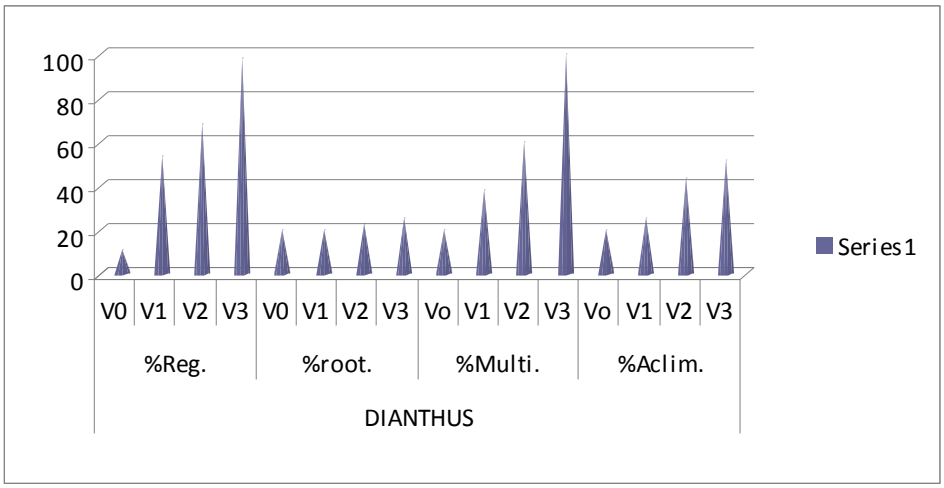

Fig. 1 The evolution of the apex of Dianthus after 40-50 days

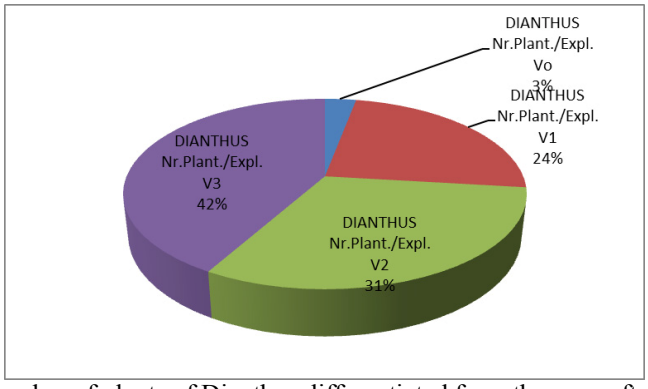

Fig. 2 The number of plants of Dianthus differentiated from the apex after 40-50 days

II. The evolution of the bud of *Drosera intermedia* Hayne. in the conditions of *in vitro* cultivation. The explant of Drosera bud has a favorable reaction to the *in vitro* culture influenced by the presence of the cytokinins in the culture medium MS. On the witness sample MS (V_o) regeneration is of 30%, but the other parameters have inferior values: on the variants with BA and Z 1 mg/l (V_1 and V_2) regeneration is of 90 and respectively 100% (Fig. 3) and the average number of neoplantlets reaches about 28-38 plantlets/explant (Fig. 4), depending on the presence of the auxin (AIB), on the nature of cytokinin (BA, Z) and also on the dose of those phytohormones.

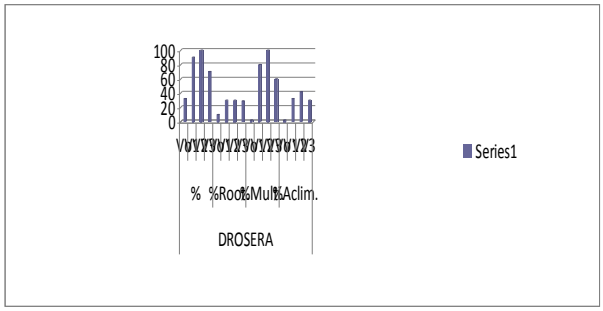

Fig. 3 The evolution of the Drosera bud after 40-50 days

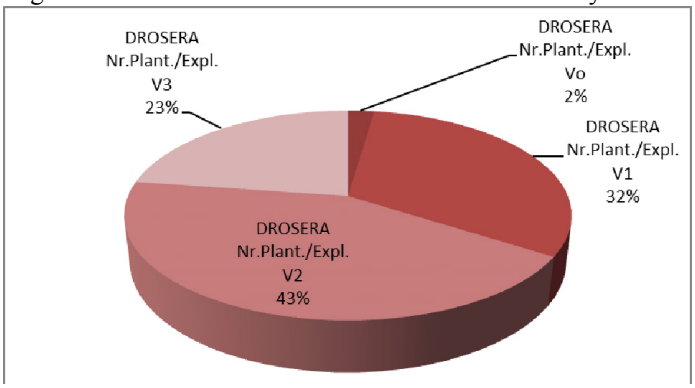

Fig. 4. The number of Drosera plants differentiated from the apex after 40-50 days

We recommend for a successful *in vitro* multiplication of the Drosera intermedia Hayne specie: the tissue formed of sections of the floral bud and the presence of the cytokinins in the basal medium in a moderate dose of about 1 mg/l (especially Zeatin), on which tissue there are formed plantlets completely organized and well rooted which acclimatize in a percentage of about 45%, acclimatization favored by a rich and vigorous Radicular system differentiated *in vitro*.

CONCLUSIONS AND RECOMENDATIONS

In vitro multiplication of the species from the spontaneous flora with the purpose of their conservation and for repopulating the natural areas, ensuring the obtaining of a large number of neoplantlets in a relatively short time, completely conformed and identical from phenotypic and genotypic point of view with the mother plant from which it was sampled the tissue. The advantage of the methods consists in the fact that for the initiation of the culture, it can be used a single explant (a single mother plant, a seed, a leaf, an apex, a meristem, a cell, etc.), without being compromised the plants from the nature that are already in a small number. The totipotency of the vegetal cell depends on the age of the explant (the younger the donor tissue, the greater the totipotency). Hence it can be used any organ, seed or part of the plant (root, leaf, stem, flower, etc.), each having their own capacity of regeneration and multiplication.

Research have proved that the rare, endangered and vulnerable spontaneous species correspond to the *in vitro* multiplication with the purpose of conserving them and of expending their areas of origin, and also in the landscape architectural spaces, based on their natural and ornamental value. Hence we conclude the following:

1. The specie cultivated *in vitro* must have exactly established the area of origin and there must be well kept the genetic variability of a population. The knowledge of the sozological category in which it fits in, the chronology and the area of propagation of the specie ensures the success of the repopulation of the protected area at the place of origin or in spaces of architectural landscape;
2. The success of the technique of *in vitro* culture depends on the specie, on its capacity to adapt to the in vitro conditions and on the capacity of rerunning the metabolism: nature, provenance and age of the explant, regenerative capacity of the tissue and the time of the year when the culture is initiated, and the climate factors from the growth chamber must be regulated according to the demands of the specie;

3. The success of the final stage of the ex vitro acclimatization process is ensured if the no./plantlets have differentiated a vigorous radicular system and if there are over passed the intermediary stages, in order to reach a percentage of survival in good free conditions;

4. In the case of *Dianthus,* the success of the multiplication of the specie is determined by the presence of the Zeatin in the culture medium in a moderate dose (1mg/l) or of the benzyl adenine (BA) in the same concentration or maybe even in a higher dose.

5. For the successful *in vitro* multiplication of the *Drosera specie*, we recommend a tissue formed of sections of the floral bud (of about 2mmØ) and the presence of cytokinins in the medium in a moderate dose of about 1 mg/l (especially Zeatin), conditions which stimulate the formation of completely organized plantlets, well rooted, which have the capacity to acclimatize in a percentage of 45%.

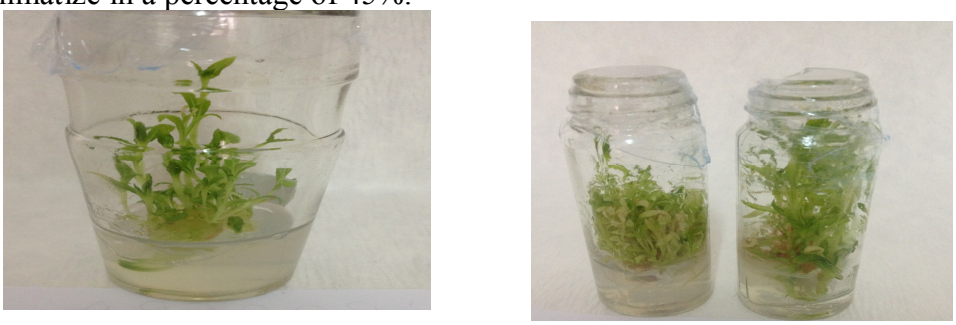

Photo.1Apex of Dianthus cultivated *in vitro*

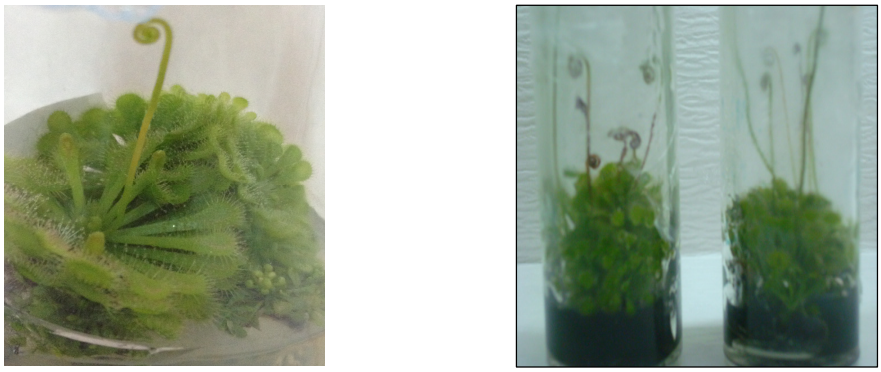

Photo.2 Bud sectioned from Drosera, cultivated *in vitro* after 50 days

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REFERENCES

1. Bajaj, Y., 1986, *In vitro* preservation of genetic resources, IAEA-SM-282/66 Vienna;
2. Banciu, C., A. Brezeanu., M. Paucă-Comănescu., 2006, Reactivitatea *in vitro* a speciei vulnerabile *Crocus flavus* Weston, Micropropagarea speciilor vegetale. în: Lucrările celui de al XV lea Simpoz. Naţi. de Culturi de Ţesuturi şi Celule Vegetale, Iaşi, 2006, p. 50-59 ;
3. Bavaru A., Godeanu S., Butnaru G şi Bogdan A., 2007, Diversitatea şi ocrotirea naturii, Editura Academiei Române Bucureşti;
4. Blându, R., I. Holobiuc, 2006, *Armeria maritima* ssp. Alpina - *ex situ* conservation using *in vitro* techniques, Acta Universitans Cibiniensis, Univ. Lucian Blaga, Sibiu ;
5. Blându, R., I. Holobiuc, 2007, Contributions ti *ex situ* conservation of rare plants from Piatra Craiului massif using biotechnology, Conference Proceedings The 1st International Conferance Enviironment – Natural Sciences – Food Industrx in European Context Ensfi 2007, 1st edition, p. 483-788;
6. Blându, R., I. Holobiuc, 2008, *Conservarea ex situ a speciilor de plante din lista roşie a plantelor superioare în România*., în: Lucrările celui de al XVI-lea Simozion Naţi. de Cult. de Ţesuturi şi Cel. Vegetale., Cluj-Napoca, Ed. Risoprint, pp. 153 – 168;
7. Bleahu M., 2004, Arca lui Noe în sec. XXI. Ariile protejate, Ed. Naţional, Buc;
8. Boşcaiu, N., Coldea G., Horeanu C, 1994, Lista roşie a plantelor vasculare dispărute, periclitate, vulnerabile şi rare din Flora României. Ocrot. Nat. Med. Înconj., 38 (1), Buc.;
9. Cachiţă, D., 1987, Metode in vitro la plantele de cultură, „Bazele teoretice şi practice, Ed. CERES, Buc.;
10. Cachiţă., D.. Ardeleanu, A., 2009, Tratat de biotehnologie vegetală, Vol. II., Editura Dacia, Cluj – Napoca;
11. Cristea, Victoria., M. Miclăuş, M. Puşcaş., C. Deliu, 2004, Conservative micropropagation of some endemic or rare species from the Dianthus L. genus. In: In vitro Cult. and Hortic. Breeding, Fifth IV CHB Symposium Biotehnology, as therorz and Practice in Horticulture, 2004, p. 3-13;
12. Cristea M., 2006, Biodiversitatea, Ed. CERES, Bucureşti ;
13. Cristea, Victoria., M. Miclăuş, M. Puşcaş., C. Deliu, A. Halmagyi., 2004, The micropropagation of some endemic or rare taxa from Gilău, M-tele Mare massif. Contrib. Bot., XXXIX, Cluj – Napoca, p. 201-209;
14. Cristea, Victoria, 2010, Culturi in vitro fotoautotrofe la speciile de Dianthus endemice şi periclitate din România, Ed. Todesco, Cluj – Napoca;
15. Cristea, V., S. Denaeyer., J.P. Herremans., I, Goia., 1996, Ocrotirea naturii şi Protecţia Mediului în România, Ed. University Press., Cluj – Napoca, p. 365;
16. Dihoru, Ghe., Alexandrina, Dihoru, 1994, Plante rare, periclitate şi endemice din flora României – Lista roşie. Acta Bot. Hort. Bucureşti, pp. 173-197;
17. Domuţa, C. (coord.), Brejea, R., Şandor, Maria, Domuţa, Cr., Borza, Ioana, Timar, A., Vuşcan., A., Ciobanu, Gh., Csep, N., Ciobanu, Cornelia., Bucureanu, Elena, Zăpârţan, Maria, Curilă, M., Romocea, Tamara, Sarca, Gh., Laslo, V., Pantea, Emilia, Samuel, Alina, Oneţ, Aurelia, Oneţ, C., Şerban, Eugenia, Costea, Monica, Gîtea, M., *Agud, Eliza***,** Bodog, Marinela, Jude, E., Cozma, Alina (2013), "*Monitoringul mediului: lucrări practice",* Ed. Universităţii din Oradea, Oradea ISBN : 978-606-10-0993-0, 607p. ;
18. Engelman F., 1991b, In vitro conservation of horticultural species. Acta horticulture, 298;
19. Engelman F., 1997, In vitro conservation methods, in: Callow, J. A., Ford-Lloyd, B. V., Newbury, H. J., (eds.) Biotechnology and Plant genetic Resources, 119-161;
20. De Langhe, E.A.L., 1984, The rol of in vitro techniyues in germoplasm conservation, în: Crop Genetic resourrce: Conserv. and Eval. Ed. Holden, J.H.W., Williams, J.T., Allen and Unwin, London, pp. 131-137;
21. Farusworth, Elizabeth (Lead Author), Sahotra Sarkar (Topic Editor). 2007, Conservation and management of rare plant species. in: Encyclopedia of Earth. Eds. Culter J. Cleveland (Washington, D.C., Environmental Information Coalition, National Council for Science and the Environment).[Published in the Encyclopedia of Earth Ahgust 28, 2007; Retrieved January 16, 2008 ;

22. Fay, M. F., 1992, Conservation of rare and endangeres plant using in vitro methods, In vitro Cell. Dev. Biol., 28P;
23. Halmagyi, A., Butiuc-Keul, A., 2007, Conservarea resurselor genetice vegetale, Ed. Tedesco, Cluj – Napoca;
24. Laslo V., Zăpârţan M., Agud Eliza - "In *vitro conservation of certain endangered and rare species of Romanian spontaneons flora",* în: Analele Universităţii din Oradea, Fascicula: Protecţia Mediului,vol.XVI A, Ed. Universităţii din Oradea, 2011a;
25. Laslo V., Vicaş S., *Agud Eliza*, Zăpârţan M., - " Methods of conservation of the plant germplasm. In vitro techniques", în: Analele Universităţii din Oradea, Fascicula : Protecţia Mediului,vol.XVI B, Ed. Universităţii din Oradea, 2011b;
26. Laslo Laslo, V., 2013, BIOTEHNOLOGII VEGETALE şi aplicaţiile lor, Ed. Univ. din Oradea, 2013;
27. Maunder, M., S. Higgens 1998, A survey of Bern Convention taxa in Europan botanic gardens – initial findings. Bot. Gard. Conservation News 2(10), p. 29-33;
28. Murashige, T., F. Skoog, 1962, A revised medium for rapid growth and bioassays with tobacco tissue culture. In: Physiol. Plant., 15, pp. 374-497;
29. Olteanu, M., G. Negreanu., A. Popescu, N. Roman., G. Dihoru., V. Sandală., S. Mihăilescu., 1994, Lista roşie a plantelor superioare din România, Studii Sinteze Documentaţii de Ecologie., Academia Română, Instit. de Biol. Bucureşti., pp. 16-31
30. Primack, R.H., 2001, „Conservarea diversităţii biologice", Ed. tehnică, Bucureşti (traducere);
31. Zăpârţan, M, 1994, The conservation of some rare and protected plants from Romania using in vitro methods, in: Vii-th INternational Congress of Plant Tissue and Cell, Culture, Firenze, June 12-17, p. 44;
32. Zăpârţan, M, 1995, Specii endemice rare şi ocrotite conservate prin tehnici de cultură *in vitro* (*Dianthus spiculifolius* Schur.), Anuarul Univ. Oradea, Biologie II, p. 42-49
33. Zăpârţan, M, 1996, Conservarea of *Leontopodium alpinum* using *in vitro* culture techiques. Bot. Garden Micropropagation News, Kew, 2, p. 26-19;
34. Zăpârţan, M, 1996 Rolul culturilor de ţesuturi în conservarea unor specii rare pentru salvarea şi extinderea lor în cultură, Contrib. Bot. Cluj – Napoca, p. 217-221;
35. Zăpârţan, M, 2001, Conservarea florei spontane prin înmulţire *in vitro*. Ed. ALC MEDIA GROUP, Cluj –Napoca;
36. Zăpârţan M**.,** Butiuc. Keul A., 2002, „ In vitro multiplication and callus induction of *Syringa josikaea Jacq*. endemic taxa from Romanian flora, în Contribuţii Botanice, XXXVII, 2002, Grădina – Botanică „Alexandru Borza" Cluj – Napoca;
37.*** Flora, Republicii Populare Române., T. Săvulescu, (ed.), de la Vol. I din 1952, până la vol. XIII. 1974
38.***IBPGR (International Broard for Plant genetic resources),1986, Design, planning and operation of in vitro genebanks, IBPGR, Rome;
39*** Conferinţa ONU de la Rio de Janeiro, 1992;
40.***Convenţia asupra biodiverstsităţii; Reţeaua ecologică „Natura 2000"
41.*** IUCN: International Union for the Conservation of Nature
42. *** UNEP:Unired Nation Environmental Programme.